MUTATIONS IN MUCORS[1]

ALBERT F. BLAKESLEE
Station for Experimental Evolution, Cold Spring Harbor, N. Y.

THE theory of mutations has played an increasing rôle in experimental evolution since its enunciation some twenty years ago. Sudden germinal changes, large or small in amount, have been the basis of perhaps the most fundamental work in modern genetics. It is natural that mutations should have been first sought for and found primarily in higher organisms, and in connection with the sexual reproduction which is characteristic of such forms. It became evident later that mutations could not be confined to cells associated with sexual reproduction, but, as shown by the somatic mutations' involved in bud sports in plants, and in similar less common phenomena in animals, they may occur in cells in which sexual processes are not involved. They have been found in lowly organized plants and animals in which nonsexual reproduction is the rule or in which sexual reproduction is not known to occur.

The mucors are a fungous group in which multiplication is brought about chiefly by nonsexual spores produced in sporangia. Sexually formed zygospores are rarely found in most forms. There are two main groups as regards their sexual reproduction:—dioecious forms and hermaphrodites. The sexual races of the dioecious forms are in the main similar in appearance, and the uniting sex cells or gametes are apparently morphologically equivalent. For this reason, the terms plus and minus have been applied to their opposite sexes instead of the terms male and female used in reference to the morphologically distinct sexes in higher forms. In many cases it has been possible to obtain a sexual reaction, called "imperfect hybridization," be-

tween plus and minus races of different species. This imperfect hybridization reaction has also been used in testing the sexual tendencies of hermaphrodites and their mutants.

It is in a species of the hermaphrodites (Mucor genevensis) that the mutations discussed in the present paper have been found. Races of this species from three different sources have been kept running in vegetatively propagated pure lines for 19 years. The species was studied in 1913 with the hope of inducing germinal changes by subjecting its vegetative growth or mycelium to various physical and chemical stimuli. Before concluding that any variation found after subjecting the mycelium to a given stimulus was in fact brought about by this stimulus, it was necessary to discover what, if any, variations the fungus would produce under normal conditions. So many variants were discovered, however, in this preliminary study, where no special stimuli were applied, that extensive investigations have not yet been attempted as to the range of variations under abnormal conditions.

METHOD OF GROWING MUCORS

The method of growing these mucors is relatively simple. To be sure that there is no doubt as to the purity of the stock with which one starts, it is desirable to obtain a culture from a single vegetative spore. This single-spore culture is grown in a test tube and, in addition to slow-germinating zygospores, produces numerous sporangia containing thousands of nonsexual spores. These sporangiospores are mixed with water and the spore mixture diluted until a platinum loop will con-

[1] A preliminary report of mutations in mucors was given in Year Book of the Carnegie Institution of Washington XII, 104–105, 1913 and presented before the Amer. Soc. of Naturalists, Dec., 1914.

SPECIMEN JARS USED IN ISOLATION CULTURES

The Dwarf true-breeding mutant is shown in the jar at the right, and on the left is shown mutant "X," an unstable mutant reverting to normal type "Y." The "Dwarf" is eleven days old "and its slowness of growth can be seen in comparison with the seven day old culture on the left. (Fig. 26.)

tain the proper number of spores as determined by examination under the microscope. The requisite number of spores are transferred with the loop to a tube of melted nutrient agar, and the agar then poured into an inverted specimen jar used as a roll tube shown in Fig. 26. By proper manipulation under the water tap, the agar is hardened, thus holding the spores uniformly scattered in a thin layer inside the tube. It goes without saying that the various steps in this process should be carried on with regard to the precautions necessary to prevent contamination with foreign spores. By the second day the spores have germinated and produced mycelial colonies which rapidly increase in size and eventually cover the available nutrient. Several hundred colonies can be readily observed in a quart sized tube.

In such an isolation culture just described, it is usual to find a few of the colonies which differ more or less in appearance from the normal growth expected for the species. The difference may be in the color or compactness of growth of the mycelium, in the lengths of the sporangium stalks, or in the size and abundance of the zygospores which are later produced, or in a

number of other characters, but most commonly in the reduced size of the colonies. The aberrant colonies may be transferred uncontaminated to test tubes before the spores are produced or their spores may be used in making a new isolation in a second roll tube. In all, somewhat over 38,000 colonies from individual sporangiospores have been inspected and a relatively large number of variants of different degrees of distinctness have been obtained. The history of nearly all of these mutants is similar in that the mutants tend eventually to revert to the normal type. Two, however, have seemed more stable than the rest.

A FIXED DWARF MUTANT FOUND

A mutant which can conveniently be called the "Dwarf" was found in March, 1913 in an isolation of spores from a single test tube culture of the normal stock. Among 1015 individuals, a single colony was noticeably smaller than the rest and different from its neighbors in the density of its mycelium and the ragged edges of its growth. Transfers of the mycelium to test tubes and continued cultivation for nearly seven years on a variety of media in gross and isolation cultures make it apparent that the mutant is fixed and constant. Its characteristic appearance, in an isolation culture, is shown in the right hand tube in Fig. 26. This is eleven days old, and its slowness of growth can be seen in comparison with the seven day old culture on the left, especially with the lower colony marked "X" which had more room for extension than its neighbors. Perhaps the most striking peculiarity of the Dwarf is its lack of definitely formed spores characteristic of the group and found in all our other mutants. The mycelium is granular and readily breaks up into bits of the mycelium, so that isolation cultures from these fragments can be made, but no sporangia have ever been observed. The difference in size of the colonies in the tube photographed may be due to a difference in the size of the fragments from which they grew. The

granular nature of its vegetative growth is merely a more pronounced expression of a tendency already present in the parent stock especially when grown in sugar solutions. Like the normal stock, the Dwarf also is able to take active part in alcoholic fermentation.

Zygospores, normally characteristic of this hermaphroditic species, are also entirely lacking, as might be expected from so weak a growth. For the same reason perhaps, it fails to give any sexual reaction with plus and minus races of a test dioecious species. The inhibiting effect upon growth of adjacent colonies may be noted in the photograph by their flattened outlines and the clear space between, where otherwise they would grow into contact. Instances where colonies seem to overlap are due to colonies showing through from the other side of the tube.

A MUTANT FROM A COLONY LACKING ZYGOSPORES

The second mutant to be considered which appears to breed true was found February 22, 1913 in a two weeks old isolation culture of 265 colonies. This single colony, labelled A1, entirely lacked zygospores which thickly dotted all the other colonies in the series. It was freed from its zygosporic neighbors, with which its sporangia were intermingled, by streaking its spores on nutrient agar in a Petri dish culture. Some of the colonies produced zygospores and were considered to be from spores of other adjacent colonies. Some were entirely free from zygospores. One of the latter was transferred to a tube culture while young and labelled A2. From A2 an isolation culture was made on March 7, and produced 4631 colonies, all of which lacked zygospores. One of these colonies (A3) was used in making another isolation culture and yielded 24 colonies again, all without zygospores. The mutant race has been continued in test tube culture since 1913 and at the present writing has reached the 16th nonsexual generation. Tube A16 is distinctly different from other races of
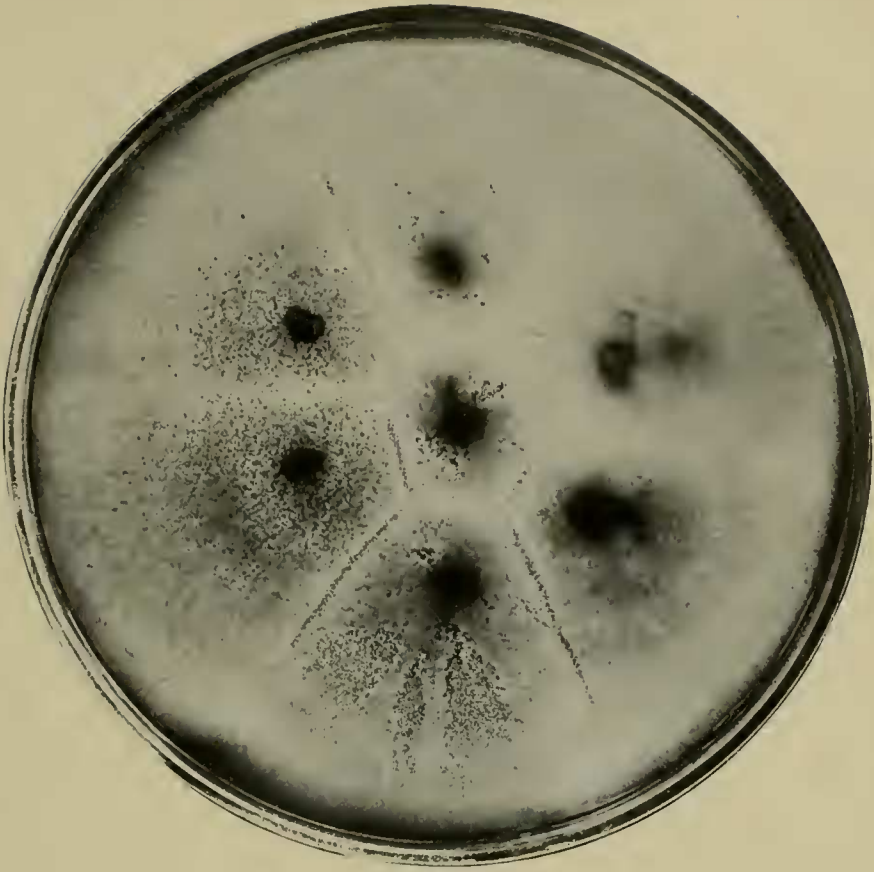
PETRI DISH CULTURE PHOTOGRAPHED BY TRANSMITTED LIGHT

The small black dots are sexual spores (Zygospores); the large dark areas are places where innoculations were made. The two left hand colonies are the normal race with numerous hermaphroditic zygospores The central row of three colonies are mutant "X" with large zygospores; the two right hand colonies are mutant "D" with only an occasional zygospore or none. Mutant "X" has a plus sexual tendency and hence forms lines of zygospores in contact with mutant "D" and the normal race, both of which have a minus tendency. (Fig. 27.)

CULTURE PHOTOGRAPHED BY REFLECTED LIGHT
The same arrangement of races as in Fig. 27 above. (Fig. 28.)

this species, primarily on account of the light appearance of growth due to the lack of zygospores. The ability to form hermaphroditic zygospores did not seem to have been entirely lost in 1913, however, since on more suitable nutrient than is available in the thin layer in an isolation tube, zygospores were occasionally produced, although in very small numbers. At the present writing (February, 1920), it fails to produce zygospores on the nutrients tested.

Tests made in 1913 of the A3 generation showed that the mutant had a minus sexual tendency since it gave good reactions with the plus races of two different dioecious species. In addition, it formed a line of zygospores with the "X" mutant known to have a plus tendency. This will be discussed in a later paragraph.

That it was not entirely lacking in the plus sex was further shown by its reaction, although weak, with a minus race of one of the dioecious test species. The mutant "A" therefore cannot be considered an example of complete transformation from a hermaphroditic into a dioecious species although it may show a tendency in this direction. It may be added that the "A" mutant has recently given rise to a striking new form "F" characterized by a low, white, felted, aerial growth and a scanty production of zygospores. It has been carried to only a few generations but so far has remained constant.

The "Dwarf" and Mutant "A" are the only examples of true-breeding mutations so far investigated in the species. Further study may show that even these have the power of reverting at times. Those discussed in the following paragraphs are examples of the more common type of reverting mutations.

MUTANTS WHICH HAVE NOT REMAINED CONSTANT

In Figs. 27 and 28 are shown two mutant races, "X" and "D," which have not remained constant under cultivation. The two colonies at the left in each figure are the normal stock; the three colonies in the central row are the "X" mutant and the two colonies at the right are the "D" mutant. The photograph shown in Fig. 27 was taken by transmitted light and shows the hermaphroditic zygospores as small black dots; the large dark areas are the places where the inoculations were made. The photograph in Fig. 28 was taken by reflected light and shows better than does Fig. 27 the differences in habit of growth between the three races.

Mutant "X" has a lower, whiter growth than the normal race. Its sporangia, as well as its zygospores, are less abundant and the latter are somewhat larger than normal and tend to be arranged in groups, which often form dark sectors radiating from the point of inoculation. Its greatest interest lies in the fact that it forms a line of zygospores with the normal race on its left as well as with the "D" mutant on the right, as shown in Fig. 27 and less well in Fig. 26. It was this ability to form lines of zygospores with adjacent colonies that attracted our attention to its first appearance in an isolation culture of a strongly zygosporic mutant consisting of 41 colonies. Ordinarily, as mentioned under the Dwarf mutant, colonies exercise some inhibitory action toward one another which retards their growth on adjacent sides and prevents their meeting when the nutrient is thin, as in an isolation culture. The inhibitory action is absent and the colonies meet when they are of opposite sexual tendencies. This seems to be the case with mutant "X" and its parent race. The normal race (called "Y") gives a strong reaction with plus test races of dioecious species and is therefore a hermaphrodite with a minus tendency, while mutant "X" gives a strong reaction with minus test races and is therefore a hermaphrodite with a plus tendency. In a similar way mutant "A" and mutant "D" have been shown to be hermaphrodites with a minus tendency. Mutant "D" formed at first a yellowish dense growth almost entirely devoid of zygospores. By continued cultivation

it seems to have lost its distinctive characteristics.

The history of the mutant "X" is given in the Table on page 284. In a series of isolation cultures in 1914 the mutant bred practically true with only three possible reversions to normal out of nearly 1500 colonies. In the 17th generation in 1916 the mutant seemed to have entirely reverted. It was regained, however, from a culture of an earlier generation and by a series of isolations its ability to throw offspring like itself was again increased. In 1917, after a few generations grown in test tube cultures, the mutant again appeared to have entirely reverted to the normal parental type and could not be regained.

At two other times in this species have mutations been observed which form lines of zygospores with the normal stock: once in an isolation culture in 1916 and once ten years earlier at the germination of the zygospores. Other mutants tested have shown a minus tendency like the parent stock.

A STRIKING MUTANT FORM

One of the most striking mutant forms appeared as a small warty colony in an isolation culture of 949 colonies. A microscopic examination showed that the colony was composed exclusively of a mass of yeast-like cells somewhat similar to those that are formed when the normal mycelium of this species is submerged in a sugar solution and takes part in alcoholic fermentation. An isolation made from this original colony gave predominatingly yeast-like colonies with only a few normal colonies. At first no filaments were found and the accumulation of yeast-like cells formed a warty mound above the surface of the agar. Often the drops of water exuding from the agar in running down the inside of the tube would carry with them the yeast-like cells of the mutant and form streaks of secondary "yeast" colonies. Later each colony gave rise to a few normal filaments, the further rapid growth of which filled the culture and covered over the warty mutants. During

April, 1913, a series of four isolation cultures were made of the "yeast" mutant, resulting in 721 "yeast" colonies to 423 early reverting colonies. The records were taken on the fourth or fifth day. Eventually even the typical "yeast" colonies reverted. During August and September 1914, an attempt was made to regain the "yeast" condition from five test tube cultures which had originally contained "yeasts." A total of 5,995 colonies were examined from these tubes, but the "yeast" mutant could not be recovered. Reversion in this mutant takes place regularly in the mycelium. In other mutants reversion is apparently more common at the formation of spores.

The "X" and the "A" mutants are of especial interest from the standpoint of sexual differentiation. On account of its freedom from zygospores and its relatively strong reaction with plus test races, mutant "A," if found alone, would appear to be an unmated minus race of some dioecious species. Its very weak reaction with certain minus races might easily be missed. If mutant "X," which is a mutant in the plus direction, had been likewise devoid of zygospores and found to conjugate with mutant "A," as it actually did, one would have felt justified in considering "X" and "A" as the mated plus and minus races of a dioecious species. It is possible that in nature dioecious races may have arisen from hermaphrodites through mutations which have carried the sexual differentiation farther than was observed in our two mutants.

Burgeff has obtained mutations in the dioecious mucor genus Phycomyces. The mucors are multinucleate, normally without cross walls in the vegetative mycelium. Mutations, he considers, affect only a part of the nuclei. The more rapid division of the normal nuclei in these mixo-chimeras, as he calls the variants, would account for the reversions which almost always take place. It is possible that our reverting mutants in the hermaphroditic *Mucor genevensis* are in fact

mixo-chimeras, and that it may be possible to obtain them in pure races as Burgeff has done in Phycomyces through the germination of the zygospores.

The individual mutants considered in the present paper are representative of many variant forms that have arisen by mutation in the nonsexually propagated races of *Mucor genevensis*. They add to the evidence, already obtained from other groups, that mutations are not restricted to processes involved in sexual reproduction.

HISTORY OF "X" MUTANT

(Y represents colonies normal to X)

September, 1913	X1 First colony of mutant X	
" "	X2 Isolation culture	X and Y types present.
" "	X3 Test tube culture of an X colony from X2	
Aug.–Sept., 1914	X4 Isolation from X3	96X : 119Y
" " "	Y5 Tube from a Y colony of X4	
" " "	X5 " " " X " " "	
" " "	Y6 Isolation from Y5	0X : 481Y
" " "	X6 " " X5	512X : 3Y (?)
" " "	X7 " " X6	625X : 0Y
" " "	X8 " " X7	316X : 0Y
	X9–X13 Series of test tube cultures	
February, 1915	X14 Isolation from X13	115X : 3Y
	X15 & X16 test tube cultures	
Feb.–April, 1916	X17 Isolation from X16	0X : 204Y
" " "	Xa12 " " X11	5X : 56Y
" " "	Xa13 " " Xa12	16X : 1Y
" " "	Xa14 " " Xa13	678X : 3Y
	Xa15–Xa17 Test tube cultures	
July, 1917	Xa18 Isolation from Xa17	0X : 207Y
" "	Xa17 " " Xa16	0X : 473Y

A RANDOM TEST IN THE THEORY OF PROTECTIVE COLORATION

FREDERICK ADAMS WOODS

UNTIL Abbott Thayer had developed the theory, no one supposed that the bright and often dazzling colors of birds and other animals were in many instances a device to render these creatures not *more* but *less* conspicuous. Remembrance of the wide introduction of camouflage and dazzle-painting during the late war will doubtless do much to convince the skeptical of the essential truths of Thayer's theories—discoveries which were in their essence optical, and did not necessarily involve learned discussions in natural history.

In Thayer's elaborate and magnificent book on the subject of protective coloration, a large number of black-and-white, and sometimes colored, pictures are presented by way of proof, but inasmuch as the authors[1] have been accused of being over-zealous in finding support for their theory, some impartially, and accidentally acquired evidence is not without interest.

Such evidence can now be supplied by museum material. In the old days, natural history museums were dismal places to visit. Stuffed animals, usually moth-eaten, were kept in dark and dusty cases, scientifically labeled and seldom seen. Now we have in many of the larger museums beautifully and accurately constructed artificial backgrounds as suitable settings for the wild life exhibited (as if in nature) and surrounded by natural objects—leaves, twigs, stones, and sand.

The four pictures, here presented, were taken in the Museum of the Bos-

[1] Thayer, G. H. and A. H., "Concealing Coloration in the Animal Kingdom," 1909.